± 16%. Thus, we considered as significant, a response giving more than a 16% increase in migration.

Results and discussion. As depicted by Figure 1, TP8 induces during its growth in Swiss B mice, the thymic alteration as well as the splenomegaly already reported by others for various transplanted syngeneic tumors[1-3,7].

Figure 2 shows the results of a typical experiment where we have compared the migration of thymocytes from normal mice in the presence of serum from mice bearing this tumor b) to the migration of the same thymocytes in the presence of serum from healthy control mice a). The mean migration area (± S.E.) for 10 capillary tubes was found to be 972 ± 25 (arbitrary units) in b) and 540 ± 12 in a), which corresponds to a 80% increase of the thymocyte migration in the serum from the cancerous mouse as compared to the migration in normal serum.

As represented by Figure 3, this thymocyte-migration-increasing effect of the serum from mice bearing TP8 depends on the weight of the tumor. The percent migration increase reaches the threshold of 16% when the tumor weighs 1 g, and then rises progressively to a maximal mean value of 55% when the tumor weighs 4 g. Subsequently, the effect seems to fall slightly. We obtained similar results using another methylcholanthrene-induced tumor.

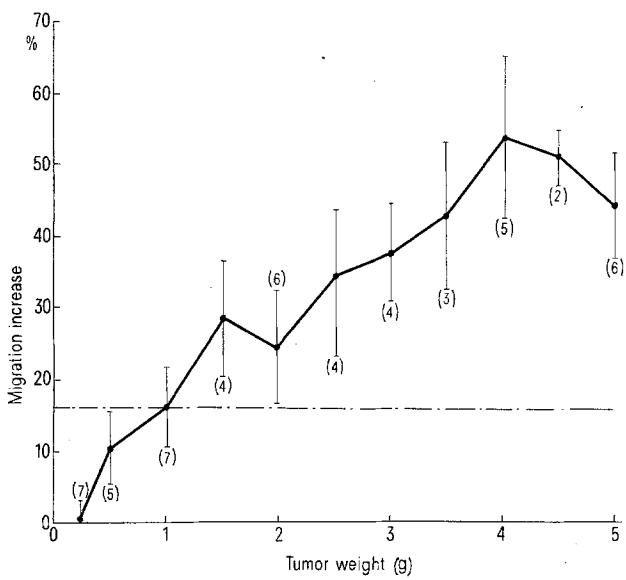

Figure 3. Percent increase of the migration of normal Swiss B thymocytes in $^1/_{10}$ diluted serum from TP8-bearing Swiss B mice as a function of tumor-weight. Each point represents the mean (± S.E.) for the number of experiments indicated in parentheses. Every experiment included at least 6 capillary tubes in the serum under test and 6 capillary tubes in control normal serum. The dotted line gives the upper limit (2.6 S.D.) of the range for 40 capillary tubes maintained in normal serum.

This action of the serum from tumor-bearing mice on the migration of thymocytes might be interpreted in 2 opposite ways: either as a true stimulation or as the suppression of an inhibition. There could exist either a thymocyte-stimulatory substance in the serum from tumor-bearing mice, or the reverse, an inhibitory one in the normal serum which would disappear when the tumor is flourishing. We have evidence in favour of this second hypothesis. We found that normal mouse serum contains some factor inhibiting the in vitro migration of syngeneic or allogeneic thymocytes. This factor is stable for at least 1 month at −20°C and can be readily destroyed by heating to 56°C for 30 min[8]. Its amount in the serum could substantially decrease when the tumor has reached a critical size, but this remains to be established.

Although the physiological meaning of these changes in the properties of the serum is still obscure, the comparison of Figure 3 with Figure 1 reveals a striking parallelism between the evolution of the action of the serum on the in vitro migration of thymocytes and the behavior of the thymus. The thymus begins to involute when the increase of in vitro migration begins to rise significantly. Moreover, the onset of this phenomenon seems to coincide with the peak of splenomegaly. It would probably be a simple view to consider the capillary tube system as an exact model of what occurs in the organism. Nevertheless, this increased motility of thymocytes in the sera from our tumor-bearing mice might be of importance in the mechanism of progressive involution of the thymus during tumor development[9,10].

Résumé. La migration in vitro, hors de tubes capillaires, des thymocytes de souris est plus importante en présence du sérum de souris porteuses d'une tumeur syngénique transplantée chimio-induite qu'en présence de sérum normal. Ce phénomène, qui est fonction du poids de la tumeur, pourrait être en rapport avec l'involution thymique accompagnant la croissance tumorale.

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[10] This work was supported by the INSERM (Contract No.: ATP72-5-495-12).

Stilboestrol-Induced Depression of the Antibody Response

Involution of the thymus is a well established consequence of administration of natural or synthetic oestrogenic compounds to experimental animals[1]. Such treatment is also known to induce a wasting syndrome in neonatal animals[2], similar to the post-thymectomy wasting[3], which appears to be partly due to increased susceptibility to infection of such animals[4]. Although administration of oestrogens has been reported to induce lymphopoenia in adult animals[5], their effects on peripheral lymphoid tissues are rather variable[1,6,7]. Reports concerning effects of oestrogens on immunity have also been somewhat contradictory. Resistance to infection in adult animals is generally increased[8], but antibody responses have been found to be unaffected[9], increased[10] or decreased[11].

In the present work we report results obtained on the humoral antibody response to sheep erythrocytes in mice and rats pretreated with stilboestrol (diethylstilboestrol).

Table I. Effect of stilboestrol on the antibody response in rats to high and low doses of SRBC

Dose of SRBC	Treatment	Parameters of the haemolysin response				Parameters of the haemagglutinin response			No. of rats
		Log_{10} peak titre	Total log_{10} titre[a]	Rate of accumulation: First and most rapid	Rate of accumulation: Average to peak titre	Log_2 peak titre	Total log_2 titre[a]: Whole antibody	Total log_2 titre[a]: 2-ME resistant antibody	
10^7	None	2.92 ± 0.06	1.93 ± 0.18	1.81 ± 0.12	1.81 ± 0.12	5.60 ± 0.25	3.43 ± 0.33	Undetectable	5
10^7	Stilboestrol	2.06 ± 0.25[b]	1.35 ± 0.15[b]	1.60 ± 0.31	1.13 ± 0.16[b]	3.16 ± 0.60[b]	1.20 ± 0.24[b]	Undetectable	5
10^9	None	3.28 ± 0.16	2.19 ± 0.18	3.27 ± 0.32	2.23 ± 0.38	5.66 ± 0.33	3.94 ± 0.33	1.80 ± 0.31	6
10^9	Stilboestrol	3.46 ± 0.14	2.37 ± 0.19	3.22 ± 0.05	1.99 ± 0.08	5.00 ± 0.37	3.83 ± 0.29	1.67 ± 0.25	6

All values are means $\pm$ s.e. [a]Average of the sum of titres on days 2, 4, 6, 9, 11 and 13 of the response. [b]Significantly less than untreated controls ($P < 0.025$ to $P < 0.001$ in the 2-sided test).

The result indicate that both the dose of antigen and the route of immunization determine whether the antibody response will be depressed after administration of stilboestrol, findings which might explain some of the inconsistencies mentioned above.

Methods. Outbred ASI female mice, average weight approximately 30 g, and outbred female albino rats, average weight approximately 200 g, were used. Stilboestrol dissolved in arachis oil was administered subcutaneously as a single injection. Mice received 1 mg in 0.2 ml, and rats 10 mg in 0.5 ml. Sheep red blood cells (SRBC) in Alsever's solution were washed and suspended in 0.9% saline and a known number was injected i.p. or i.v. (via a tail vein) into mice in a volume of 0.5 ml and intravenously (via a tail vein) in 1.0 ml into rats. Animals pretreated with stilboestrol were immunized 3 days later.

All animals were bled under ether anaesthesia, mice from the orbital venous plexus and rats by cardiac puncture, and sera were inactivated by heating and stored at −80°C until titration. All titrations were made on individual sera. Haemolytic antibody was titrated using the 50% end-point method[12]. Haemagglutinin titrations were performed in the presence or absence of 2-mercaptoethanol (2-ME)[13]. Statistical analysis of the results after logarithmic transformation was carried out using Student/Welch's *t*-test[14].

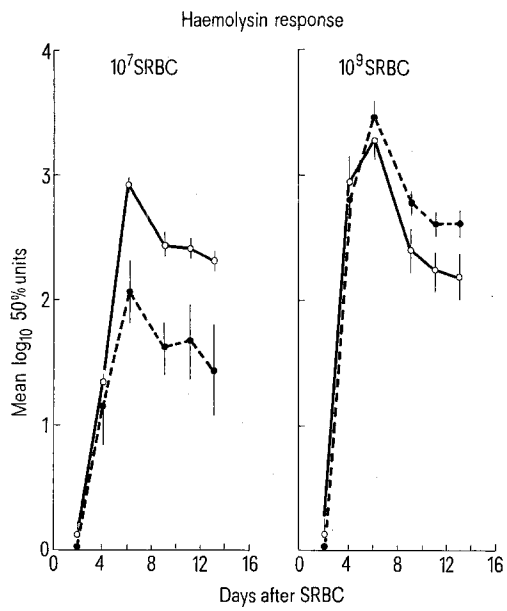

Fig. 1. Mean initial haemolysin responses in control rats (○—○) and rats pretreated with stilboestrol (●—●) immunized intravenously with 10^7 or 10^9 SRBC.

Results. Antibody response in rats. The time course of the haemolysin response to 10^7 and 10^9 SRBC is shown in Figure 1. Pretreatment with stilboestrol markedly depressed the response to the lower dose of antigen, but was without effect in rats immunized with the larger dose. Results on the effect of stilboestrol treatment on the various parameters[12] of the haemolysin and haemagglutinin antibody responses calculated for individual animals are given in Table I. The amount of haemolytic and haemagglutinating antibody produced, as measured by the peak titre and the total titre (i.e. the average of the sum of titres over the period of time under experiment), was reduced in rats immunized with 10^7 SRBC. The average rate of haemolytic antibody accumulation was also reduced in these animals, although the initial rate of accumulation as well as the length of the latent period and the length of the rise to peak period (not shown in the Table) were not affected. None of the parameters of the 2 antibody responses were altered in rats pretreated with stilboestrol and immunized with the larger dose of 10^9 SRBC.

Antibody response in mice. The depressive effect of stilboestrol on the antibody response was also demonstrable in mice immunized with 10^7 and 10^8 SRBC. In addition to the dose of antigen used, stilboestrol-induced

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Table II. Effect of stilboestrol, antigen dose, and route of immunization on the antibody response to SRBC in mice[a]

Treatment			Total $\log_{10}$ haemolysin titre	Total $\log_2$ haemagglutinin titre	
Dose of SRBC	Route	Stilboestrol[b]		Whole antibody	2-ME resistant antibody
10^7	i.v.	—	1.38 ± 0.14	3.04 ± 0.18	1.68 ± 0.15
10^7	i.v.	+	0.82 ± 0.18[c]	2.00 ± 0.24[c]	1.50 ± 0.19
10^8	i.v.	—	1.86 ± 0.17	4.23 ± 0.28	3.29 ± 0.42
10^8	i.v.	+	1.44 ± 0.17	3.78 ± 0.30	2.41 ± 0.27
10^8	i.p.	—	2.01 ± 0.18	3.58 ± 0.35	3.44 ± 0.23
10^8	i.p.	+	0.90 ± 0.15[c]	2.34 ± 0.24[c]	2.34 ± 0.25[c]

[a] All values are means ± s.e. of individual titres on days 3, 5, 7, 10, 12 and 14 of the response for groups of 5 AS1 female mice. [b] Stilboestrol (1 mg) was given 3 days before antigen. [c] Significantly less than untreated controls ($P < 0.02$).

depression appeared to depend on the route of immunization. The amount of antibody produced in response to the lower dose of antigen given i.v. was reduced in terms of both haemolysins and haemagglutinins, but the response to the larger dose of antigen given by the same route was not affected after treatment with stilboestrol (Table II). On the other hand stilboestrol-treated mice immunized with 10^8 SRBC i.p. produced significantly less antibody, as detected by the 2 methods, including the 2-ME resistant antibody which is considered to be IgG. Representative average antibody response curves shown in Figure 2 illustrate that administration of stilboestrol can, under appropriate circumstances, result in a marked depression of the response to SRBC.

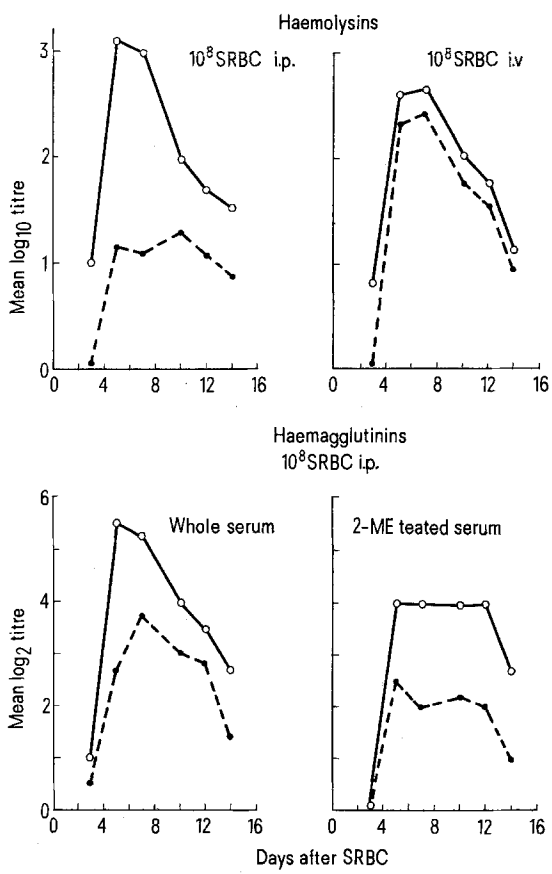

Fig. 2. Mean initial haemolysin and haemagglutinin responses in control mice (○—○) and mice pretreated with stilboestrol (●—●) immunized intraperitoneally or intravenously with 10^8 SRBC.

Discussion. A survey of the reports available (cf. [15], and above) indicates, in addition to the variability of observed effects of oestrogens on the antibody responses, that in the great majority of cases multiple injections of hormones were used and that the studies were limited to a single dose of antigen and route of immunization. The present results show that these 2 latter factors can be of importance in determining the effects of oestrogens on the antibody response to a given antigen. The importance of the dose of antigen used to induce an antibody response was clearly shown in boths rats and mice. In both species the antibody response to a low dose of antigen could be readily depressed by pretreatment with a single dose of stilboestrol. No depression was, however, demonstrable after a high dose of antigen, which may account for some of the previous failures to show immunodepressive properties of oestrogens. The present results also indicate that the antibody response is more easily depressed by stilboestrol when the antigen is administered intraperitoneally than when the same dose of antigen is given i.v. The significance of this is not quite clear, although it may be related to the reduced amount of a particulare antigen such as SRBC reaching the site of antibody formation as a result of being trapped in the peritoneal cavity (unpublished observations).

The mechanism of the immunodepressive effect of stilboestrol is not clear at present and several possibilities could be considered. In view of the fact that SRBC is a so-called thymus-dependent antigen [16] and that oestrogens cause marked thymic involution it could be speculated that this is a possible mechanism for the observed stilboestrol effect.

It has been reported that oestrogens prevent the proliferation and maturation of cells of the granulocytic and lymphocytic series in the bone marrow and that, under condition of recovery from irradiation, they also impair the restoration of the immunological capacity [7]. It is therefore possible that in normal animals interference of oestrogens with cells of bone marrow origin might be responsible for the immunodepressive effect found in the present study.

The last possibility concerns the well established effects on the phagocytic activity of the reticuloendothelial system of many oestrogenic compounds, among which

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stilboestrol is one of the most potent[8]. Through such an effect stilboestrol may alter the distribution and/or processing of the antigen resulting in a reduced antibody response. This would be in contrast to the adjuvant effect found for a number of other substances which equally cause a marked increase in the phagocytic activity of the reticuloendothelial system[17,18]. If the depressive effect of stilboestrol was due to antigen redistribution it is consistent with this explanation that its effect should be overridden by a larger, presumably saturating, dose of antigen. This and other possibilities for the mechanism of action of oestrogens on the antibody response are being currently investigated.

Résumé. On a étudié l'effet du stilboestrol sur la formation d'anticorps contre de hématies de mouton chez les rats et les souris. Un traitement préliminaire au stilboestrol affaiblissait chez les rats et les souris la réponse exprimée en termes d'anticorps hémolytiques et agglutinants. On a montré que cet effet dépendait de la dose et de la voie d'administration de l'antigène. On discute brièvement les mécanismes susceptibles d'expliquer cette diminution.

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Immunological Evidence for the Homogeneity of an Ovine Pituitary Glycoprotein with Dual Gonadotropic Activity

The isolation, from ovine pituitaries, of a homogeneous glycoprotein exhibiting both FSH[1] and LH activities was reported in a brief communication from this laboratory[2]. This protein (P1–2) was monodisperse when examined by such physico-chemical techniques as ultra-centrifugal analysis, disc electrophoresis and gel electro-focusing. We wish to report briefly immunological evidence in support of the homogeneity of this protein.

Immunization of rabbits was carried out according to the procedure of Moudgal and Li[3], using the crude Koenig-King extract[2] as the antigen[4]. Following periodic bleeding and testing for antibody titre, the antiserum was tested against crude extract, P1–1, as well as P1–2, using the agar gel diffusion method of Ouchterlony and also micro-immuno-electrophoresis[5]. In addition, the antiserum was tested against HCG, PMS, ovine FSH and ovine LH.

The antiserum raised against the crude extract gave precipitin reactions with the crude extract, P1–1, P1–2 and ovine FSH, respectively, but not with HCG or PMS, when tested by the agar gel diffusion method (Figure 2). At least 2 precipitin lines were present in the reactions with the crude extract, as well as with P1–1 and ovine FSH.

[1] The abbreviations used are: FSH, follicle stimulating hormone; LH, luteinizing hormone, HCG, human chorionic gonadotropin; PMS, pregnant mare serum gonadotropin.
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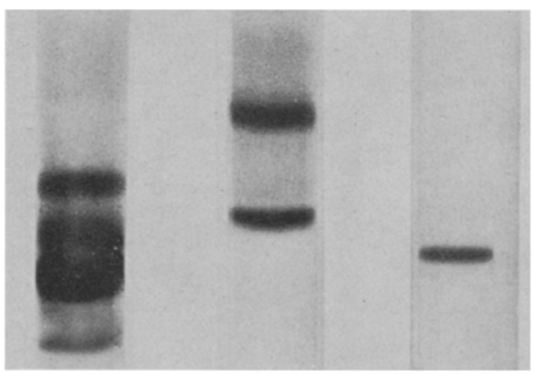

Fig. 1. Acrylamide gel electropherograms of (left to right): crude Koenig-King extract, P1–1 and P1–2, respectively, stained with Amido Black. Length of gels 5.5 cm; pH (*Tris* buffer) 8.6; migration towards bottom (anode).

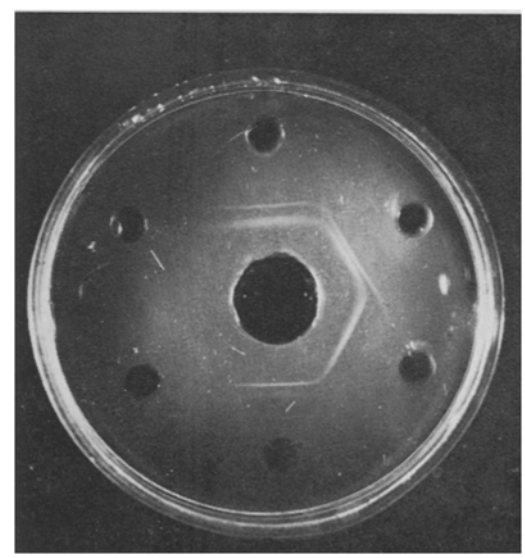

Fig. 2. Ouchterlony diffusion pattern: Antiserum to crude extract in the centre well. Antigens (moving clockwise from the peripheral well at top): Crude extract P1–1, P1–2, ovine FSH (NIH-FSH-S7), HCG and PMS. With ovine FSH, a faint second line was obtained. Note the single line with P1–2.